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(54) ANIMAL DEFICIENT IN MUSASHI 2 PROTEIN GENE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a nonhuman animal or its progeny which is deficient in function of Musashi 2 protein gene.
SOLUTION: The animal deficient in Msi2 (Musashi 2) gene, or the animal deficient in Msi2 and Msi1 gene is useful for elucidation of function of Msi1 and Msi2 in cells of endocrine system, and useful as the model animal for hyperglycemia, hypoglycemia and diabetes mellitus, and used for screening for remedy for these diseases.

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CLAIMS

[Claim(s)]
[Claim 1] The nonhuman animal to which the function of Musashi protein 2 gene suffered a loss, or its descendant.
[Claim 2] The animal according to claim 1 which is that to which the function of Musashi protein 1 gene is furthermore missing.
[Claim 3] The animal according to claim 1 to which the function of Musashi protein 2 gene suffers a loss by one part of Musashi protein 2 genes carrying out deletion, or inserting other genes in one part of Musashi protein 2 genes.
[Claim 4] The animal according to claim 2 to which the function of Musashi protein 1 gene suffers a loss by one part of Musashi protein 1 genes carrying out deletion, or inserting other genes in one part of Musashi protein 1 genes.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a transgenic nonhuman animal useful to assessment of the remedy of the hyperglycemia, a hypoglycemia, and diabetes mellitus, a mechanism break through of these diseases, etc.
[0002]
[Description of the Prior Art] Many transcription factors which function in growth or differentiation of a nerve precursor cell are identified. However, discovery of nerve cell specific RNA binding protein in recent years is raising possibility that generating of the nerve cell from a precursor cell is controlled, also in the phase of posttranscriptional control. Stabilization of mRNA or control by translational control is included in these. Nerve cell RNA binding protein is discovered by both non-vertebrate and vertebrate, and these are equivalent to two sorts of gene families. (Okano, Dev.Growth Diff.37:619-629 (1995)) . One is an Elav family, it is discovered by the nerve cell after fission, and the member of this family is considered to function in survival or differentiation of a nerve cell (Akamatsu et al., Proc.Natl.Acad.Sci.USA 96:9885-9890 (1999)). The Musashi (Musashi:Msi) family which is another family is discovered mainly by the nerve precursor cell on the Elav family and the contrast target (Sakakibara et al., Dev.Biol.176:230-242(1996); Pincus et al., Ann.Neurol.43:576-585(1998); Kaneko et al., Dev.Neurosci.22:139-153 (2000)).
[0003] Moreover, it is continuously discovered also after Msi1 remarkable protein becomes an adult (Sakakibara and Okano, J.Neurosci.17:8300-8312 (1997)). Thus, about the Musashi protein 1 (Musashi1;Msi1), cloning is carried out among the Msi families which have played the role important for generating and maintenance of a nerve cell (Sakakibara et al., Dev.biol.176:230-242 (1996)). however -- the Musashi protein 2

(Musashi2:Msi2) which is another type in the mammals -- us -- the existence -- suggesting -- **** -- it does not pass, and the function is not clear, either and cloning is not carried out, either. Then, as a result of examining many things that cloning of the Msi2 gene should be carried out, from the lambda gt11 cDNA library obtained from the adult-mice cerebellum, this invention person succeeded in cloning and did patent application previously (application for patent 2001-250186). However, about the function of Msi2 in an adult, it is hardly known, but waits for the break through.

[0004]

[Means for Solving the Problem] Then, when this invention persons examined what kind of manifestation pattern Msi1, Msi2, and Numb would show in the pancreas of adult mice, it became clear that it was discovered on the whole ten gel HANSU island where, as for both Msi1, Msi2, and Numb, an endocrine cell exists also unexpectedly. Then, when Msi2 knockout mouse, Msi1, and a Msi2 double knockout mouse were produced, these knockout mice are carrying out the abnormality manifestation of the glucagon with the insulin in the spleen, and it came to complete a header and this invention for it being useful as a model animal of the hyperglycemia, a hypoglycemia, and diabetes mellitus.

[0005] That is, this invention offers the nonhuman animal to which the function of Msi2 gene suffered a loss, or its descendant. Moreover, this invention offers the nonhuman animal to which the function of Msi1 gene and Msi2 gene suffered a loss, or its descendant.

[0006]

[Embodiment of the Invention] this invention persons are the RNA affinity protein which carried out cloning for the first time, and the mouse Msi2 in this invention has the amino acid sequence shown by the array number 1 or 2. Here, the array number 2 has the amino acid sequence in which 18 amino acid (264-281) in the array number 1 carried out deletion. These two sorts are the isoforms of Msi2. The array number 1 is called Msi2L and the array number 2 is called Msi2S. Msi2 has two RNA joint motifs (RRMs), and these RRM has RNP-1 which is the array well saved between RNA binding protein, and RNP-2.

[0007] Msi2 gene has the base sequence which carries out the code of the amino acid sequence shown by (1) array number 1 or 2. As the base sequence, the base sequence shown by the array numbers 3 and 4 or 5 is mentioned. In addition, the array numbers 3 are all the arrays of Msi2 gene, the array number 4 is the coding region of Msi2L, and the array number 5 is the coding region of Msi2S.

[0008] Msi2 gene can prepare a cDNA library using the cerebellum of a vertebrate, for example, a mouse, and can obtain it by the approach of choosing a desired clone from this library using a suitable probe and a

suitable antibody peculiar to this invention gene [Proc.Natl.Acad.Sci., USA., 78, 6613(1981);Science, 222, 778 (1983), etc.]. The approach of screening Msi2 gene from a cDNA library is not restricted especially, either, but can follow the usual approach. The plaque hybridization using the approach of choosing the cDNA clone which corresponds by immunity-screening which specifically used the specific antibody of this protein to the protein (Msi2) produced by cDNA, and the probe selectively combined with the target DNA array, colony hybridization, these combination, etc. can be illustrated.

[0009] Msi1 gene can be obtained by aforementioned Sakakibara et al., Dev.Biol., and 176:230-242 (1996). The amino acid sequence of Msi1 and the base sequence of Msi1 gene are shown in the array number 6.

[0010] In this invention, producing Msi2 or Msi1 Mr. protein which says that Msi2 which is the gene product of this gene with "the function of a gene suffered a loss", or Msi1 is not produced normally, suffers a loss in that Msi2 or Msi1 the very thing is not produced and a part, and cannot discover a function is included.

[0011] In order to obtain the functional deficit animal of Msi2 gene of this invention, after carrying out cloning of these genes and making the function of this gene suffer a loss by in vitro one, return this deficit gene to an animal, homologous recombination is made to cause between Msi2 genes on a chromosome, Msi2 gene on a chromosome is destroyed, and, generally the approach of making the function of the animal itself or this gene of the descendant suffer a loss is used. Moreover, in order to obtain the functional deficit animal of Msi2 and Msi1 gene, it is obtained by making the Msi2 functional deficit animal created by the above-mentioned approach cross with the existing Msi1 functional deficit animal (JP,2001-17027,A).

[0012] The approach of adding variation to a gene artificially as an approach of making the function of a gene suffering a loss, and destroying this gene being mentioned, for example, inserting or permuting a part of [at least] deletion of promoterregion and/or a coding region and other genes is mentioned.

[0013] any of the animal to which, as for the nonhuman animal as used in the field of this invention, the function of Msi2 gene or Msi2, and Msi1 gene is [that what is necessary is just also ******** / Msi2 gene or Msi2, and Msi1 gene] missing to the animal which is missing to the hetero, and the gay -- although -- it is contained. Moreover, it is not limited, but all the animals except Homo sapiens are mentioned, especially the animals used are mammalians, such as a guinea pig, a hamster, a mouse, a rat, a rabbit, and Buta, preferably, and its rodent with a comparatively short biocycle with easy and treatment, especially mouse as a symptoms model are desirable.

[0014] As the technique of introducing [technique] a gene into an animal and making the individual or descendant of the animal discover the gene The

well-known technique regularly used by creation of a transgenic animal from the former can be mentioned. For example, the approach of pouring Gene DNA into the pronucleus term germ of a fertilized egg, the method of infecting a recombination retrovirus with an early embryo, The host germ obtained by the approach of pouring into 8 blastocyst or cell term germ the embryonic stem cell (embryonic stem cell) which made homologous recombination causing etc. is transplanted to an animal, offspring is obtained, this is crossed with other individuals, and F1 hetero variation animal and the approach of creating F2 gay or a hemi variation animal further are mentioned. Among these, since the approach of transgenics using an embryonic stem cell has the advantage that it is suitable for destroying a gene by homologous recombination (knockout), and can carry out by dividing the process which introduces a gene into an embryonic stem cell, and the process which creates a chimera animal, it is desirable. What is necessary is just to perform the approach of transgenics using an embryonic stem cell according to a well-known approach.

[0015] Hereafter, about the approach of installation of the gene using an embryonic stem cell, a mouse is made into an example and explained concretely. What is necessary is to carry out deletion of a part of promoterregion and/or coding region [at least] to the deficit of the Msi2 gene function of a mouse, or just to insert other genes in one of parts. Moreover, as long as the function of these genes can be made to suffer a loss, the part which inserts deletion or other genes may be the intron. And in performing homologous recombination between Msi2 genes, DNA (targeting vector) which has the DNA array built so that a gene might be destroyed in this way is produced.

[0016] It is desirable to use the gene which functions as a marker gene for detecting the deficit of Msi2 gene as a gene to insert, and for example, a thymidine kinase (tk) gene, a diphtheria toxin A fragmentation (DT-A) gene, etc. are used as a marker gene used for positive sorting as such a gene as a marker gene which for example, a neomycin (neo) resistance gene uses for negative sorting. In addition, a neomycin resistance gene enables sorting of the object gene by using G418 which is a neomycin analog.

[0017] The approach using the inserting type vector which controls the gene expression etc. is mentioned by inserting the non-region of homology to which a positive sorting marker is equivalent to the backbone of the vector which includes a selective marker for the approach using the permutation mold vector permuted on the target gene, and the upstream of a target gene as a targeting vector as a desirable gene targeting. In addition, a DNA recombination technique in ordinary use can perform insertion of these genes by in vitro one.

[0018] Next, homologous recombination is performed between the targeting

vector obtained in this way and Msi2 gene in an embryonic stem cell. Electroporation in ordinary use can perform installation to the embryonic stem cell of DNA for homologous recombination. In this homologous recombination, recombination arises between the fields where DNA of Msi2 gene in an embryonic stem cell and DNA for homologous recombination correspond, and the marker gene inserted into DNA for homologous recombination is inserted in Msi2 gene of the genome of an embryonic stem cell. Consequently, an embryonic stem cell will suffer a loss in the function of Msi2 gene, and will contain a marker gene simultaneously. Based on the sorting function of this marker gene, the embryonic stem cell which suffered a loss in the function of Msi2 gene can be sorted out.

[0019] Next, this embryonic stem cell is poured into host germs, such as a blastocyst of a mouse, the obtained germ is transplanted to the uterine horn of a pseudopregnancy mouse, and a chimeric mouse is obtained. The offspring of a terrorism mold is obtained to F1 by crossing this chimeric mouse with the mouse of a suitable system. If the reproductive cell of a chimeric mouse originates in the embryonic stem cell by which homologous recombinant, i.e., Msi2 gene, is destroyed, the mouse with which the function of Msi2 gene suffered a loss can be obtained. Moreover, the obtained hetero deficit animals can be made to be able to cross and a gay deficit animal can be obtained out of the offspring.

[0020] It can be checked [the deficit of Msi2 gene, and] by performing Southern blotting or PCR after pouring out DNA from a tail whether this gene suffers a loss to a hetero or a gay, after resulting in ablactation.

[0021] In addition, the breeding approach of the animal of this invention does not need to use a special approach, and can breed it by the same approach as a normal animal.

[0022] In the same symptom as the human hyperglycemia or diabetes mellitus, for example, the pancreas, the animal to which the function of Msi2 gene [which was obtained by this invention] or Msi2, and Msi1 gene suffered a loss presents the abnormality manifestation of glucagon with an insulin, as the after-mentioned example shows. Therefore, the animal of this invention can turn into the hyperglycemia, a hypoglycemia and a diabetic model animal, and an animal for a mechanism break through of these disease generating.

[0023]

[Example] Next, although an example is given and this invention is further explained to a detail, this invention is not limited to these examples.

[0024] A. An ingredient and an approach (1) The lambda gt11 cDNA library (Sakakibara et al., Dev.Biol.176:230-242 (1996)) obtained from the cloning adult-mice cerebellum of the mouse msi2 gene cDNA Mouse msi1 gene coding region The EcoRI fragment and platanna xrp1 of 1.1 kilobase pairs of

(Genbank bitter taste session number #D4965, Sakakibara et al., and Dev.Biol.176:230-242 (1996)) The BamHI-NdeI fragment of 387 base pairs containing a cDNA (Genbank bitter taste session number #L02953, Good et al., Nucl.Acids.Res.21:999-1006 (1993)) coding region carboxyl terminus It used and screened. To 1x107 plaques, using the msi1 radioactive probe, hybridization is 60 degrees C and was performed using the xrp1 radioactive probe in the buffer solution (1M sodium chloride, 1%SDS, 10% dextran sulfate, 0.1mg [/ml] salmon sperm DNA) which is 55 degrees C and contains 18 to 24 hours, and the random 5x105cpm [/ml] ply MUDOPU lobe by which 32P indicator was carried out. The filter by which hybridization was carried out was washed twice for 20 minutes at the room temperature in 2xSSC and 0.1%SDS (low stringency). 32 electropositive clones hybridized to both msi1 gene and the xrp1 radioactive probe were obtained. nine pieces strongly hybridized to xrp1 cDNA in it are chosen, and subcloning is carried out to pBluescriptII (Stratagene, La Jolla, California) -- having -- and a die primer kit (Amersham Pharmacia Biotech, Buckingham Shache, Britain) -- using -- a law -- the base sequence was determined by the die deoxy nucleotide sequence method of a method. In sequence analysis, two or more duplication clones crossed to 5'3 of untranslation region and 0.8 kilobase pairs' untranslation region of the 0.5 kilobase pairs named the mouse musashi2 (msi2) and the presumed open reading frame (ORF) of 1.0 kilobase pairs became clear. The exon on presumption by which selection splicing is carried out was discovered by the carboxyl terminus of a prediction coding region as insertion of 54 base pairs, and formed ORF of the short form of msi2 cDNA, and long form. The manifestation by in vivo of the msi2 imprint object of short form and long form was checked in the reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of RNA isolated from the brain of E12 fetus and an adult. Similarity retrieval and alignment were performed by the NCBI server using BLAST and a FASTA algorithm. The bitter taste session number of the protein used for genealogical tree analysis (clustalW program on the WWW server of DDBJ) is as follows. Hu(Homo sapiens) U1snRNP70K (A25707), Mus(mouse) hnRNP A1 (NP034577), Mus hnRNPA2 [Hu hnRNP A0 (Q13151) and]/B1 (O88569), Hu hnRNP A3 (P51991), a Hu hnRNP A/B type (AAA36575), Rat AUF1 (BAB03468, BAB03466, BAB03467), Mus hnRNP C1/C2 (AAD03717), Hu hnRNP F (S43484), Mus hnRNP G (O35479), Hu hnRNP H (I39358), Mus PTB (hnRNPI) (P17225), Mus brPTB (NP062423), Hu hnRNP L (P14866), Hu hnRNP M (P52272), Hu hnRNP R (T02673), Mus hnRNP U (NP058085), Mus TIA-1 (P52912), Mus TIAR (S72436), Mus HuR (NP034615), Mus HuB (AAC52644), Mus HuC (Q60900), Mus HuD (JC2298), Hu Brunol3 (AAB09040), Mus Lark (NP033058), and Rat La/SS-B (JC1494).

[0025] (2) The ICR (CD-1) mouse used for adjustment of an animal and an

organization adjustment organization protein extract, RNA, or an organization intercept was purchased from Charles River Japan Inc. (Japan). The pregnancy day was determined by existence of a vaginal plug, was recorded as 0th day (E0) of viviparity, and set the birth day to P0.
[0026] (3) Total RNA (20microg) was isolated using Trizol (Gibco-BRL, Grand Island, New York State) according to a manufacturer's instructions from northern-blot-analysis each mouse organization and an embryo. It dissociated by the electrophoresis of 1% agarose-formaldehyde gel, and transferred on the biotechnology dyne B nylon film (Pall, Portwasington, New York State). 32P indicator probe of 3' untranslation region fragment of mouse msi2 cDNA 750 base pair was adjusted using the random PURAIMUDO DNA indicator kit (Roche Diagnostics, Mannheim, Germany), and was hybridized at 42 degrees C for 16 hours in 50% formaldehyde, 6xSSPE, 5x DIN heart liquid, 0.5%SDS, and the 200microg [/ml] salmon sperm DNA. It washed strictly at 50 degrees C in 0.1xSSC and 0.1%SDS after the incubation, and the filter was exposed on the Kodak X-OMAT film for 48 hours. The integrity of the transferred RNA sample checked by carrying out the re-probe of each blot using a radiolabel beta actin probe (Clontech, Palo Alto, California).
[0027] (4) It compounded for antibody production of the peptide with which the cysteine amide residue equivalent to production of anti-Msi2 antibody and the terminal sequence (MEANGSPGTSGSAN) of 14 amino acid of purification Msi2 protein continues. This peptide array does not overlap the array of RRM1 which is an RNA joint domain, and does not indicate any similarity to be the N terminal region to which Msi1 protein corresponds. About 15mg peptide was combined with the keyhole limpet hemocyanin (KLH) by which m-BUROMO succinimide processing was carried out through cysteine amide residue, and it was used in order to give immunity to New Zealand SHIROUSAGI. 2-fluoro which activated the synthetic peptide (5mg) according to a manufacturer's instructions in order to carry out affinity purification of the anti-Msi2 antiserum - Covalent bond was carried out to 1-methyl pyridinium-toluene-4-sulfonate (FMP) SERURO fine (Seikagaku Kogyo, Japan). It incubated at 4 degrees C with 3ml of peptide-FMP SERURO fine affinity resin which equilibrated in advance 10ml of all antisera that carried out filter filtration (0.45 micrometers) using the TBS buffer solution (0.15M sodium chloride, 20mM tris hydrochloride, pH7.5). 50ml of after that 1M sodium chlorides, 1% Triton X-100, and a tris hydrochloride -- resin was continuously washed 20 mM (pH 7.5) using 20ml of 0.15M sodium chlorides, and it was eluted at 4 degrees C using 4ml (pH 2.0) of 100mM glycine hydrochlorides, and neutralized by 1M tris 0.2ml promptly.
[0028] (5) The BamHI-EcoRI fragment of 983 base pairs and 1072 base pairs equivalent to ORF of the short form of recombination Msi1 and Msi2 protein Msi2 protein and long form was isolated from RNA of the brain of E12 fetus

and an adult by RT-PCR. Subcloning was carried out to the in frame at expression vector pRSET-A (Invitrogen, Carlsbad, California), pRSET-Msi2S (short form) and pRSET-Msi2L (long form) was built, and the fusion protein which has 6 histidine residue in an amino terminus generated. Expression vector pRSET-Msi1 (Sakakibara et al., 1996), pRSET-Msi2S, and pRSET-Msi2L were introduced into the BL21(DE3) pLysS Escherichia coli stock, and the fusion protein was guided by carrying out an incubation at 30 degrees C for 6 hours using 1mMIPTG (isopropyl-beta D-thio galactopyranoside). The Probond resin (Invitrogen) column was used for the recombination fusion protein (His6-Msi2S, His6-Msi2L, and His6-Msi1) as the supply manufacturer directed, and it carried out affinity purification. The purity and concentration of a fusion protein checked the SDS polyacrylamide (PAGE) gel of an eluate with the Coomassie Brilliant Blue (Sigma) dyeing and the Bradford assay (Biorad, Heracles, California).

[0029] (6) Protein phosphatase processing and the immunity blotting method organization extract were homogenized using the buffer solution A (50mM tris hydrochloride pH7.6, 1mM potassium acetate, 1.5mM magnesium acetate, 2mM dithiothreitol (DTT), 100microg [/ml] phenylmethyl sulfonyl fluoride (PMSF), 5microg [/ml] aprotinin, 5microg [/ml] leupeptin), and they carried out at-long-intervals alignment separation in 10,000xg continuously for 10 minutes. It dissociated by SDS-PAGE gel 10%, and the electro blot of the purification recombination protein (50ng / lane) or the organization extract (amount [of 30micro] of protein g / lane) discovered bacteria was carried out to the Immobilon-P film (Millipore, Bedford, Massachusetts) using semi dry imprint equipment. It confirmed that the equivalent quality of total protein was loaded from each organization with standard Bradford assay, and it proved by carrying out Coomassie blue dyeing of the duplex duplicate gel. According to directions of a manufacturer, ECL (Amerasham Pharmacia Biotech) detected the chemiluminescence signal using the Kodak X-OMAT film. In order to perform protein dephosphorization measurement, Msi2 protein of internality carried out partial purification from the fetus brain extract (E12.5). Centrifugal separation of the brain (it is 1.0g at wet weight) of E12.5 was homogenized and carried out to buffer-solution A5ml, and the nucleus was settled (for 12,000xg and 10 minutes, 4 degrees C). Supernatant liquid was removed, on the cushion of 1.5ml of cane sugars (it is w/v 30% in the buffer solution A), multistory was carried out calmly and centrifugal separation was carried out for 2 hours using the BeckmanSW55Ti rotor at 130,000xg and 4 degrees C. After removing S130 supernatant liquid and a cane-sugar cushion, the polysome fraction which precipitated was rinsed and it re-suspended in ejection and buffer-solution A 500microl. Most quantity of Msi2 protein was collected from this polysome fraction. a protein phosphatase processing sake -- 10micro of protein g of the purification

polysome fraction origin -- 50mM tris (pH7.5) of 25microl, 0.1mM EDTA, 5mM DTT, the 0.01% bridge 35, 2mM manganese chloride, and 10microg/ml It incubated at 30 degrees C for 1 hour with the lambda protein phosphatase (lambdaPPAse) (New England BioLabs, Beverly, Massachusetts) of 800 units in PMSF, 5microg [/ml] aprotinin, and 5microg [/ml] leupeptin. lambdaPPAse carries out dephosphorization of the serine by which phosphorylation was carried out, threonine, and the thyrosin residue. The contrast sample which does not contain lambdaPPAse also incubated to above-mentioned **. It stopped using the SDS-PAGE sample buffer solution, and the reaction was separated in SDS-PAGE gel 10% about the immunity blotting method.

[0030] (7) The cDNA fragment equivalent to the coding region (base of No. 1564 [524 to]) of an in vitro imprint / translation, and RNA joint assay Msi2 long form and the coding region (the base, the bitter taste session number D49654 of No. 1152 [64 to]) of Msi1 was isolated by PCR using the primer which carries out the code of the FLAG tag to an amino terminus. Subcloning was carried out to pCDNA3 (Invitrogen), and expression vector pCDNA-msi2 and pCDNA-msi1 were built. These plasmids follow the conditions which a manufacturer recommends in a rabbit reticulocyte solution (TNT T7 Quick coupled imprint / translation system, Promega, Mathison, Wisconsin), and are 0.4 mCi/ml. It was made to imprint/translate under 35S methionine (Amersham Pharmacia Biotech) existence. The luciferase T7 control vector (Promega) which carries out a code also made 61kilodalton luciferase protein translate as above-mentioned by invitro. in Although some modification was added about association to the RNA homopolymer of the protein by which the vitro translation was made, as indicated fundamentally before, it carried out (Swanson and Dreyfuss, 1988). if it states briefly -- the joint buffer solution (10mM tris hydrochloride, pH7.4, and a 2.5mM magnesium chloride --) 0.5% Triton X-100 or 2mg [/ml] pepstatin, 2mg [/ml] leupeptin, The RIBOHOMO polymer-agarose bead of 20microeach I which equilibrated by the aprotinin and 1mg [/ml] heparin 0.5% with the protein (1x105cpm) by which 35S indicator was carried out In the joint buffer solution of 500microl containing the sodium chloride of 100mM(s) or 250mM(s), it incubated at 4 degrees C on the shaking table for 15 minutes. The bead precipitated by the short-time revolution, and before re-suspending in the 50microl SDS-PAGE loading buffer solution, 500micro of joint buffer solutions I washed it 5 times. You made it eluted by boiling, it dissociated in SDS-PAGE 10%, and the united protein was made to visualize with fluorography.

[0031] (8) The fragment of XbaI of 4.4kbs which exist in the upstream of the first exon containing the initiation codon of Mis1, and the EcoRV-Ecl136I fragment of 3.9kbs which exist in the lower stream of a river of the second exon including the field which carries out the code of the RRM-A field

down-stream were tied to the upstream of the construction neomycin resistance gene of a targeting vector, and the targeting vector designed so that DNA might be permuted by the neomycin resistance gene by two homology recombination in the 102 amino acid of Msi2 gene was used (drawing 1 and 2).

[0032] (9) By carrying out transfection of the production targeting vector of a chimeric mouse, the embryonic stem cell (-four shares of 129 SvJ/RW) which permuted Msi2 locus of one of the two by the neomycin resistance gene was injected into the germ cell which was made to cross C57BL/6 mouse with F(C57BL/6xDBA)1 mouse, and was obtained, and the chimera animal was made. The male chimera originating in each embryonic stem cell stock was made to cross with C57BL/6 female, and heterozygote F1 descendant was made born. F1 heterozygotes were crossed and the Msi2-/-mouse was acquired.

[0033] B. the stringency which used the mouse msi1 and the Xenopus xrp1 cDNA probe, and was reduced for the purpose of isolation of cDNA of the identification and the property Msi2 of the primary structure of result (1) Msi2 -- a mouse -- a nervous system -- the cDNA library was screened. Xenopus xrp1 gene (Good et al., Nucl.Acids Res.21:999-1006 (1993)) is carrying out the code of the protein related with NRP1 protein which is the platanna homologue of Msi1 (Skakaibara et al., Dev.Biol.176:230-242 (1996)) on an array. In order to obtain an overall length cDNA, the gained longest DNA was used as a probe for screening a cDNA library with severe stringency. The longest and only open reading frame which carries out the code of the protein of 346 amino acid with a prediction molecular weight of 37kilodalton was identified from nine duplicate cDNA(s). In array analysis, it became clear that the gene product by which a code is carried out to cDNA is new RNA binding protein. We named this Msi1 related gene musashi2 (msi2). It was shown that two sorts of transcripts by which the selection splice was carried out exist by carrying out RT-PCR analysis about the msi2 imprint object of the RNA origin isolated from two or more cDNA clones obtained from library screening and the mouse brain of E12 and an adult. These two sorts are divided by existence of the short segment in the carboxy end one half of Msi2 (18 amino acid), and nonexistence. It is shown that the isoform of prediction molecular weight 36.9 and two sorts of Msi2 35.7kilodalton (Msi2L and Msi2S were named respectively) protein generates this selection splice. The amino acid sequence of Msi2L was shown in the array number 1, and the amino acid sequence of Msi2 was shown in the array number 2. Moreover, the base sequence of msi2L was shown in the array number 4, and the base sequence of msi2S was shown in the array number 5.

[0034] (2) Although the property Msi2-/-mouse of a Msi2 genetic-defect

mouse was macroscopically normal, in Langerhans' islet of the pancreas, abnormalities were in the localization of an alpha cell and a beta cell. (Drawing 3: The beta cell which green dyed using the antiinsulin antibody is shown, and the alpha cell which red dyed using the glucagon antibody is shown.) Although Langerhans' islet of a wild type shows the structure where an alpha cell covers a beta cell lump's outside as shown in drawing, in a Msi2-/-mouse, the beta cell has discovered glucagon with the insulin.
[0035] (3) the mice of property Msi1+/- (JP,2001-17027,A) and Msi2+/- (Msi1, 2 double hetero) of Msi2 and a Msi1 genetic-defect mouse are crossed -- making (drawing 4) -- Although the Msi1-/- and Msi2-/-(Msi1, 2 double gay) mouse was produced by 1/16 of establishment when Mendel's laws were followed, as for the Msi1-/- and Msi2-/-mouse, only seven animals were produced to all 164 populations produced actually. This was about 1/23, there was little establishment by which a Msi1-/- and Msi2-/-(Msi1, 2 double gay) mouse is produced a little as compared with Mendelism, and death was guessed in fetus. The born individual also presented the modality of cyanosis, and did not drink mother's milk, but died in several hours after birth (drawing 5). Moreover, the Msi1-/- and Msi2-/-(Msi1, 2 double gay) mouse had abnormalities in the localization of an alpha cell and a beta cell in Langerhans' islet like the Msi2-/-mouse (drawing 3). Compared with the Msi2-/-mouse, symptoms, like an alpha cell enters into a beta cell lump were also seen, and, as for the Msi1-/- and Msi2-/-(Msi1, 2 double gay) mouse, more serious abnormalities were seen.
[0036]
[Effect of the Invention] Msi2 gene of this invention or Msi2, and a Msi1 genetic-defect animal are useful in order to solve the role in the endocrine system cell of Msi1 and Msi2, they are useful also as model animals, such as hyperglycemia, a hypoglycemia, and diabetes mellitus, and also applicable to screening of these disease remedies.
[0037]
[Layout Table]
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[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing SUTORADEJI (first half) of targeting vector construction.
[Drawing 2] It is drawing showing SUTORADEJI (continuation of the second half and drawing 1) of targeting vector construction.
[Drawing 3] It is drawing showing the manifestation of the insulin in pancreas Langerhans' islet of a Msi2-/-mouse, and glucagon. wild: Wild type, msi 1 ko:Msi1-/-, msi 2 ko:Msi2-/-, msi 1, 2 ko:Msi1-/-, and Msi2-/- (double gay)
[Drawing 4] It is drawing showing SUTORADEJI of Msi1 and Msi2 genetic-defect mouse creation.
[Drawing 5] It is drawing showing the gestalt of Msi1 and a Msi2 genetic-defect mouse.

[Translation done.]